Embryology of *Ageratum conyzoides* L. and *A. fastigiatum*

R.M. King & H. Rob. (Asteraceae)

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ABSTRACT

*Ageratum* has a complex circumscription, and recent studies have indicated its polyphyletism. The genus has been placed in the tribe Eupatorieae whose embryology is not fully known. Embryological data are conservative and important indicators of phylogenetic relationships and can improve family relationships. This study presents, for the first time in Eupatorieae, embryological data for *Ageratum conyzoides* and *A. fastigiatum*. Both species have common features of the family such as a unitegmic anatropous ovule, basal placentation, secretory tapetum, *Polygonum* megagametophyte, and *Asterad* embryogenesis. The data obtained reinforce the heterogeneity of the family embryology and show, for the first time, the anther wall development of the monocotyledonous type for Asteraceae. The species studied show also differences between themselves. *A. conyzoides* has bisporangiated and introrse anthers, conspicuous pappus, and cypselae with trichomes on the ribs, whereas *A. fastigiatum* has tetrasporangiate and latrorse anthers, pappus absent at maturity, and glabrous cypselae. The data presented support recent phylogenetic molecular studies, suggesting the replacement of *A. fastigiatum* to another genus along with Gyptidinae.

Keywords: Compositae, embryogenesis, Eupatorieae, ontogeny, phytomelanin

Introduction

*Ageratum* comprises approximately 29 species (King & Robinson 1987) in the Americas and adjacent West Indies, and *A. conyzoides* is a pantropical introduced weed (Johnson 1971). The species name is derived from the Greek α (=not) and γερας (=old age) due to the longevity of their flowers (Johnson 1971 therein). The genus can be recognized by a conical receptacle, leaves with large glandular punctuations, large anther appendages, and cypselae with distinct and contorted carpodria (King & Robinson 1987). *Ageratum* is the best known genus in the tribe Eupatorieae (King & Robinson 1987), and its members have been cultivated in Europe since the seventeenth century as an ornamental species, while some species are used in traditional medicine to treat a variety of diseases (Johnson 1971 therein).

The delimitation of the genus *Ageratum* is complex and it has been discussed since the seventeenth century, mainly based on pappus morphology. In the current circumscription, this genus comprises species with pappus that have a distinct five-dome or cup format and connate scales (Johnson 1971). Hattori (2013), studying the phylogeny of several subtribes inside Eupatorieae, observed *Ageratum* in two separate terminals, suggesting a new combination of *A. fastigiatum* to another genus.

The embryology of Asteraceae is heterogeneous and does not show a fixed structural pattern that separates the family from other angiosperms (Johri et al. 1992). Embryology in Eupatorieae is focused only on the fruit, seed (Pandey & Singh 1983; Marzinek & Oliveira 2010; Marzinek et al. 2010), and female gametophyte development (Holmgren 1919; Bertasso-Borges & Coleman 2005).

Embryological data are constant for genera, making them useful in determining taxonomic relationships within families, genera, or species (Palser 1975; Stuessy 2009). Thus, this study aims to investigate the embryology of *A. conyzoides* and *A. fastigiatum*, thereby testing the phylogenetic hypothesis of Hattori (2013) with an ontogenetic approach.

Materials and methods

Flower buds, flowers, and fruits in various stages of development from *Ageratum conyzoides* (Gardner) R.M. King & H. Rob. and *Ageratum fastigiatum* L. were collected in Uberlândia, Minas Gerais, Brazil (19°10’942"S, 48°23’61”W and 19°11’026"S, 48°23’804”W). The voucher was incorporated into the *Herbarium Uberlândense* (HUFU) under accession numbers 20,142 for *A. conyzoides* and 57,943 for *A. fastigiatum*.

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Samples were fixed in FAA50 (Johansen 1940), stored in 50% alcohol (Berlyn & Miksche 1976), and embedded in methyl methacrylate-based. The material was 2–8μm thick on a rotary microtome. Sections were stained with toluidine blue at pH 4.7 with acetate buffer (O’Brien et al. 1964 modified), and mounted with synthetic mounting media. The slides produced were analysed and documented on Olympus BX51 light microscope.

The results were described following Marzinek & Oliveira (2010), while embryo development description was based on Johri et al. (1992).

Results

Microsporangium

In the early stages, the anther is formed by homogeneous meristematic cells surrounded by the protoderm (Fig. 1A). The cells of the primary parietal layer has hypodermic origin. They divide periclinally resulting in two secondary parietal layers. The outer secondary parietal layer differentiates directly into the endothecium. The cells of the inner secondary parietal layer undergo periclinal divisions forming the middle layer (externally) and tapetum (internally) (Fig. 1B).

The protoderm differentiates in the epidermis persisting at maturity and its cells are periclinaly elongated. Endothecium cells are also elongated and radially thickened (Fig. 1C). The middle layer is ephemeral. The tapetum has cells with dense cytoplasm and many fused nuclei (Fig. 1D). During development, the tapetum is projected toward the anther locule involving the microspores (Fig. 1D–F). Styloid crystals are observed in all layers of the developing anther (Fig. 1F). At the stage of pollen dispersal, only the epidermis and endothecium remain. Anther dehiscence of A. fastigiatum is latrorse (Fig. 1G) and is introrse in A. conyzoides (Fig. 1H).

Ageratum conyzoides has two functional abaxial sporangia, whereas the adaxial sporangia are rarely presented (Fig. 1B) and A. fastigiatum has four functional sporangia (Fig. 1F).

Microsporocytes undergo simultaneous meiosis generating tetrahedral tetrads (Fig. 1D–E) and each microspore produces a tricellular pollen grain with elongated gametes (Fig. 1I) and evident exine (Fig. 1J).

Ovary

The ovary is inferior bircarpelar, syncarpous, and unilocular (Fig. 2A–B). The outer epidermis is uniseriated with trichomes on the ribs only in A. conyzoides (Fig. 2C–D).

The mesophyll has two regions (Fig. 2E–F). The outer two layers of mesophyll have bulky (Fig. 2E), slightly longitudinally elongated cells (Fig. 2F). The internal mesophyll has four to six layers of varying diameters (Fig. 2E) and longitudinally elongated cells (Fig. 2F). Five procambial bundles accompany the ribs (Fig. 2B–D). The inner epidermis is uniseriate (Fig. 2E–F). At the base of the ovary, there is a protuberance with parenchyma cells that have various shapes constituting the carpodiwm. In both species, the carpodiwm is asymmetric (Fig. 2A, G, H).

Ovule

The ovule is anatropous, unitegmic, and tenuinucellate with basal placentation (Fig. 2A). The outer integument epidermis is uniseriate with cuboidal, juxtaposed cells and an evident nucleus. The mesophyll comprises approximately nine layers of cells (Fig. 2I–J). A procambial bundle crosses through the raphe and extends to the middle portion of the anti-raphe. The inner epidermis has one to two layers of cells with dense cytoplasm, large nuclei, and distinct nucleoli constituting the endothecium (Fig. 2J).

The nucellus has only one archespordial cell differentiating directly into the megaspore mother cell, just below the epidermis (Fig. 2K). The megaspore mother cell undergoes meiosis, giving rise to a linear tetrad of megaspores (Fig. 2L), and only the chalazal megaspore remains functional (Fig. 2M). The embryo sac is monosporic and 7-celled or a Polygonum type megagametophyte. The nucellus remnants occur around the embryo sac (Fig. 2N).

Fertilization, embryo, and endosperm

Fertilization is micropylar with pollen tube entering into the one synergid (Fig. 3A). The egg cell and one of the gametes join to form the zygote (Fig. 3B). The other gamete merges with the fused nuclei of the middle cell forming the first cell of the endosperm (Fig. 3A). The first cell of the endosperm divides forming walls between the nuclei (Fig. 3B–D). The zygote undergoes a transverse division generating basal and apiical cells (Fig. 3C). Basal cell undergo transverse division and each gives rise to two daughter cells (ci and m) (Fig. 3D). The ci cell is transversely divided into n and n’ and divides again giving rise to n, an o, and p cells, resulting in the suspensor (Fig. 3E–H). The m cell undergoes two longitudinal divisions giving rise to the quadrant m that will in turn give rise to the hypocotyl and radicle (Fig. 3D–G). The apical cell undergoes three longitudinal divisions forming the octant q, responsible for formation of cotyledons, epicotyl, and plumule (Fig. 3D–G). After successive divisions the embryo has a globular shape (Fig. 3H). The mature embryo is axial and occupies the whole seminal chamber (Fig. 3I). The embryo axis is straight, and the plumule is poorly differentiated.

Fruit and seed

During fruit development, the outer epidermis develops the exocarp, the mesophyll develops in mesocarp, and endocarp originates from the inner epidermis of the ovary.

The exocarp remains unchanged. The outer mesocarp exhibits increased cells with large vacuoles. Between the

outer and inner mesocarp, there is a partial separation of the cells forming a space schizogenous with projections of inner mesocarp connecting the two layers. The schizogenous space is filled by the secretion of phytomelanin. The inner mesocarp is formed of only one fibre layer, while the other layers were crushed by the seed growth (Fig. 4A–E).

At maturity, both the carpopodium (Fig. 4F–G) and the floral disk (Fig. 4H–I) show lignification. The pappus is lacking in *A. fastigiatum* and persistent in *A. conyzoides* (Fig. 4H–I). The exotesta shows no modifications. The internal mesotesta and endotesta are crushed (Fig. 4C–E). The endosperm cells are persistent, with one to three cell layers surrounding the embryo (Fig. 4E).

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**Figure 3.** Longitudinal sections of the seeds of *Ageratum fastigiatum* (A–D, F–I) and *A. conyzoides* (E). A–H Immature seeds. A Fertilization of egg cell and middle cell, noted synergid increased density of the cytoplasm and polar nuclei of middle cell fused (arrow). B Early zygote and first cell of the endosperm. C Proembryo resulting from the transversal division of the zygote resulting in an apical cell (ca) and basal cell (cb). D Embryo with four cells, noted q cells originated from a longitudinal division of the apical cell (ca), m and ci resulting from a transverse division of the basal cell (cb). E–F Embryo with 8 cells showing two cells q, two m cells, n and n’ derived from a transverse division of ci. G Embryo with 16 cells, which 8 q cells, 4 m, 2 n, o and p result of transversal division of n’ cell. H Globular embryo with suspensor. I Mature seed showing embryo with embryo axis straight and two convex planes cotyledons. co cotyledon, ea embryo axis, er endosperm, ga gamete, mc middle cell, oo egg cell, pl plumule, su suspensor, sy synergid, zy zygote
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**Discussion**

In *A. fastigiatum* and *A. conyzoides*, the outer secondary parietal layer originates directly into the endothecium and the inner develops a middle layer and tapetum. This pattern of anther wall development is the monocotyledonous type and is the first record for Asteraceae. In the family, the pattern described until now has been dicotyledonous (Davis 1966; Johri et al. 1992; Gotelli et al. 2008; Liu et al. 2011).

Both species of *Ageratum* present an endothecium with radial thickening. In Eupatorieae, the same type of thickening was observed as in *Eupatorium cannabinum* (Dormer 1962). The presence of crystals in the tapetum, as found in the studied species, is poorly explored in embryological studies of the Asteraceae family and has been previously reported only in *Helianthus* (Meric & Dane 2004). The presence of crystals does not necessarily represent patterns in different taxonomic levels (Buss Jr & Lersten 1972; Prychid & Rudall 1999; De-Paula & Sajo 2011).

According to Davis (1966), tetrasporangiate anthers are common in the family. *A. fastigiatum* presented tetrasporangiated anthers, and *A. conyzoides* presented bisporangiated anthers. The position of the anther opening also differs between the two species. In *A. fastigiatum*, the anther is latrorse but is introrse in *A. conyzoides*.

The tapetum cells of both *Ageratum* species protrude toward the anther locule and possess fused nuclei. Multinucleated amoeboidal tapetum is the most common type in Compositae (Johri et al. 1992); it has also been observed in Carduioideae (Yeung et al. 2011), Senecioneae (Pullaiyah 1983; Lakshmi & Pullaiyah 1987), Gnaphalieae (Davis 1962a; Pullaiyah 1979), Astereae (Davis 1968), Anthemideae (Davis 1962b; Li et al. 2010), Inuleae (Pullaiyah 1979), and Heliantheae (Gotelli et al. 2008). Secretory tapetum occurs in Pertyeae (Kapil & Sethi 1962), Cichorieae (Sood et al. 2000; Yurukova-Grancharova et al. 2006), and Vernonieae (Tiagi & Tamni 1963).

Simultaneous cytokinesis was recorded for both species of *Ageratum* and according to Davis (1966), is considered the main type of cytokinesis in the family.

As previously described for the family, both species have an anatropous and unitegmic ovule with basal placentation (Davis 1966; Johri et al. 1992).
Ageratum conyzoides and A. fastigiatum present the monosporic Polygonum megagametophyte type, which has been observed in Eupatorium (Holmgren 1919; Bertasso-Borges & Coleman 2005) and for most Asteraceae species (Davis 1962a; Johri et al. 1992). The bisporic Allium type was found in Gnaphalieae, Anthemideae, Astereae, and Heliantheae: the Adoxa type in Heliantheae, the Drusa type in Gnaphalieae, Anthemideae, and Astereae, and the Fritillaria type in Heliantheae (Davis 1966).

The cellular endosperm was observed in the species studied and is consistent with findings for the tribe (Pandey & Singh 1983; Marzinek & Oliveira 2010), it has also been found in Senecioneae (Pulliaih 1983; Lakshmi & Pulliaih 1987), Gnaphalieae (Davis 1961; 1962a), Anthemideae (Davis 1962b), Inuleae (Pulliaih 1979), and Tageteae (Misra 1964). Nuclear endosperm was found in Mutisieae (Devi 1957), Cichorieae (Yurukova-Grancharova et al. 2006), and Vernonieae (Sharma & Murty 1978). The Asteraceae tribe presents both types of endosperm development: nuclear in Erigeron bonariensis and cellular in Felicia bergeriana (Sharma & Murty 1978) and Brachycome ciliare (Davis 1964).

The embryogenesis of both species is the Asterad type, in which basal and apical cells participate in the formation of the embryo as previously described for Compositae (Davis 1966; Johri et al. 1992).

The pericarp of A. conyzoides and A. fastigiatum follows the pattern of the development observed in Eupatoriaceae, with the outer mesocarp presenting cells and large vacuoles and a phytomelanin layer and fibres (Marzinek & Oliveira 2010; De-Paula et al. 2013). Phytomelanin was observed in some tribes of Asteraceae such as Carduoideae (Pandey & Singh 1982), Vernonieae (Basak & Mukherjee 2003; Loeuille et al. 2013), Gnaphalieae (Davis 1962a), Anthemideae (Aguado et al. 2011), Heliantheae (Maheshwari & Srinivasan 1944), and Eupatoriaceae (Pandey & Singh 1983; Marzinek & Oliveira 2010). According to De-Paula et al. (2013), the fibres of the pericarp are directly responsible for secreting phytomelanin in Praxelis diffusa; this fact will probably also be true in Ageratum, where phytomelanin is observed primarily near the fibres.

In both species studied, vascular bundles correspond to the salient region of fruit called ribs in Eupatoriaceae, and both species are able to have bundles without ribs. Reinforcing the taxonomic importance for this feature, the ribs of A. conyzoides possess trichomes, and A. fastigiatum has glabrous ribs. Another important feature is the pappus, present in A. conyzoides and lacking at maturity in A. fastigiatum. The presence or absence of the pappus shows an important feature for a new circumscription of the genus.

Carpopodium promotes fruit abscission at the dispersal. Haque & Godward (1984) associated the asymmetry of carpopodium with presence of the pappus, but this was not observed in this study in which A. fastigiatum features a symmetrical carpopodium and the pappus is absent; A. conyzoides has a weakly asymmetric carpopodium and persistent pappus.

The results of this study confirm the heterogeneity of embryological processes in the Asteraceae family. Common features to the family, but not exclusive, have been observed as the unigeneric anatropous ovule, basal placentaation, secretory tapetum, Polygonum megagametophyte type, and embryogenesis of the Asterad type. Other features expressed the homogeneity of embryological data within the tribes, such as the radial thickening of the endothecium and development of the pericarp typical for Eupatoriaceae. The monocotyledonous anther development type reported for the Ageratum species studied here, and not yet described for Asteraceae, is emerging as an important feature for the tribe. Because this was the first study to describe this type of development for the family, more studies are needed to assess the true potential of this feature for Eupatoriaceae. Both species showed distinguishing features: A. fastigiatum with a tetrasporangiate and latrorse anther and A. conyzoides with a bisporangiate and introrse anther. In addition to the anther, the presence of the pappus and trichomes in the ribs can separate A. conyzoides from A. fastigiatum, corroborating the phylogenetic hypothesis of Hattori (2013) that suggests that Ageratum should be segregated into two distinct clades: one with A. conyzoides, which is widely distributed in the Americas, and one with A. fastigiatum, which, along with Gyptidinae, is exclusively distributed in Brazil.

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References

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